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# Immature Stages of Selected Meliponine Bees (Apoidea: Apidae)

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## ABSTRACT

This paper describes the eggs and last larval instars of certain species of bees belonging to the tribe Meliponini, one of the four related tribes that comprise the corbiculate bees in the subfamily Apinae. The four taxa analyzed include some whose immature stages have previously been described. Our purpose is to identify what is known about the anatomy of immature stages and suggest what needs to be studied to better understand the developmental anatomy of eggs and mature larvae in this group of highly eusocial bees.

## INTRODUCTION

This is the fifth of a recent series of studies undertaken by the first author to explore the egg anatomy, its hatching process, and the last stage larvae of corbiculate bees, including the Euglossini (Rozen, 2018), Bombini (Rozen et al., 2018a, 2018b), Apini (Rozen et al., 2017), and this study of Meliponini, each members of the Apidae. These studies are far from complete; the goal is to focus on what is known to date, with the hope that future investigations can be better directed.

Following a statement on methods, eggs of selected Meliponini are described, with extensive comparative description of final larval instars. Details pertaining to both eggs and larvae of the tribe are briefly discussed.

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## METHODS

Most of the immature stages studied herein were initially preserved in Kahle's solution. For study, preserved larval specimens were cleared in an aqueous solution of sodium hydroxide and stained with an ethanol solution of Chlorazol Black E, and then photographed with a Canon Power Shot A2300 camera, hand held to one of the eyepieces of either a Leitz Wetzlar stereomicroscope or a Carl Zeiss compound microscope. SEM micrographs of eggs of *Melipona beecheii*, *Melipona panamica, Scaptotrigona pectoralis*, and *Tetragonisca angustula* were captured with a Hitachi S5700 SEM in the Microscopy and Imaging Facility of the American Museum of Natural History. All scale bars in illustrations equal 1 mm. Nomenclature and order of presentation below mostly follow Michener (2007).

## EGGS OF MELIPONINI

The known eggs of this tribe often appear to be nearly symmetrical around their long axis. However, "lateral views" in many cases seem to present a somewhat different image from that of a dorsal or ventral view. Such apparent inconsistencies may be real or may result from postmortem manipulation of preserved specimens, for example by forceps. In the following presentation, both symmetrical as well as asymmetrical views are shown without judgment as to which is correct.

All eggs described and illustrated here are considered to be reproductive eggs and not trophic eggs because of the well-developed reticulate pattern on their chorions (Pereira et al., 2006).

### *Melipona* (*Melikerria*) *beecheii* Bennett

Figures 1, 2, 9–13

Because only a single, reasonably well-preserved egg was available, diagrams of it could not be modified to eliminate possible asymmetries resulting from preservation procedures, hence, the slight asymmetry in the dorsal view of the egg (fig. 1). Furthermore, it is uncertain whether the egg is symmetrical in lateral view (fig. 2), although the developing embryo within (dotted outline) clearly is not. That part of the embryo shown is the developing head, partly embedded in the swollen prothorax, with its labrum touching the chorion.

The eggs of the two species of *Melipona* described here (figs. 1–4) are larger and differently shaped than those of the other two meliponine species (*Scaptotrigona pectoralis* and *Tetragonisca angustula*) treated herein.

Diagnosis: The larger size as well as the distinctive shape of the egg of this species and that of *M. panamica*, described below, distinguish them from those of the other two genera.

Description: Color white; chorion smooth and not lustrous under low magnification; clear chorion on one specimen with strongly expressed hexagonal surface pattern even when viewed without SEM. Shape, based on single specimen as diagrammed, elongate as seen in dorsal view (figs. 1, 9), greatest width at approximate midlength dorsal view; outline narrowing gradually from there

toward both ends; extreme anterior end a blunt curve viewed dorsally (fig. 1); micropyle nearly flush with surface, appearing as small funnellike depression on middle of anterior end (figs. 10, 11); posterior end tending to constrict more than the anterior before expanding at extreme rear end. Dimensions: length 2.5 mm; greatest width at approximate midlength 1.15 mm dorsal view.

When viewed with SEM (figs. 9–13), chorion of middle part of egg as revealed in upper part of figure 13 with strong pattern of elevated hexagonal ridges; on anterior end of egg, those hexagons becoming elongate and teardrop shaped as they approach centrally positioned micropyle, viewed in figure 10 and close-up in figure 11; micropyle circular with pores central (fig. 12); chorion at posterior end of egg lacking hexagonal ridges (fig. 13).

Material Studied: Larva with partly attached chorion: Mexico: Yucatán: Xmatkuil, January 17, 2018 (J. Quesada).

## *Melipona* (*Michmelia*) *panamica* Cockerell

Figures 3, 4, 14–18

The morphology of the *M. panamica* eggs was essentially identical with that of *M. beecheii* and supported the identification on the label. The shape of the egg of *M. panamica* is questionably symmetrical along its long axis. Although specimens seem to have a less exaggerated form in one view than in another, this might be the result of either postmortem manipulation or a real asymmetry.

Description: Color white; chorion smooth and not lustrous under low magnification; clear chorion with strongly expressed hexagonal surface pattern at anterior end and absent at posterior end. Shape elongate as diagrammed as seen in presumed dorsal view (fig. 3), greatest width at approximate middle, narrowing gradually from there toward both ends; extreme anterior end variably rounded (figs. 3, 4); micropyle projecting slightly (fig. 15); posterior end increasing in size (figs. 3, 4, 14, 18), appearing rounded before ending. Dimensions: length 2.5–2.6 mm; greatest width measured at right angle to long axis at approximate midlength 1.125–1.2 mm; greatest width of posterior expansion 0.925–0.95 mm.

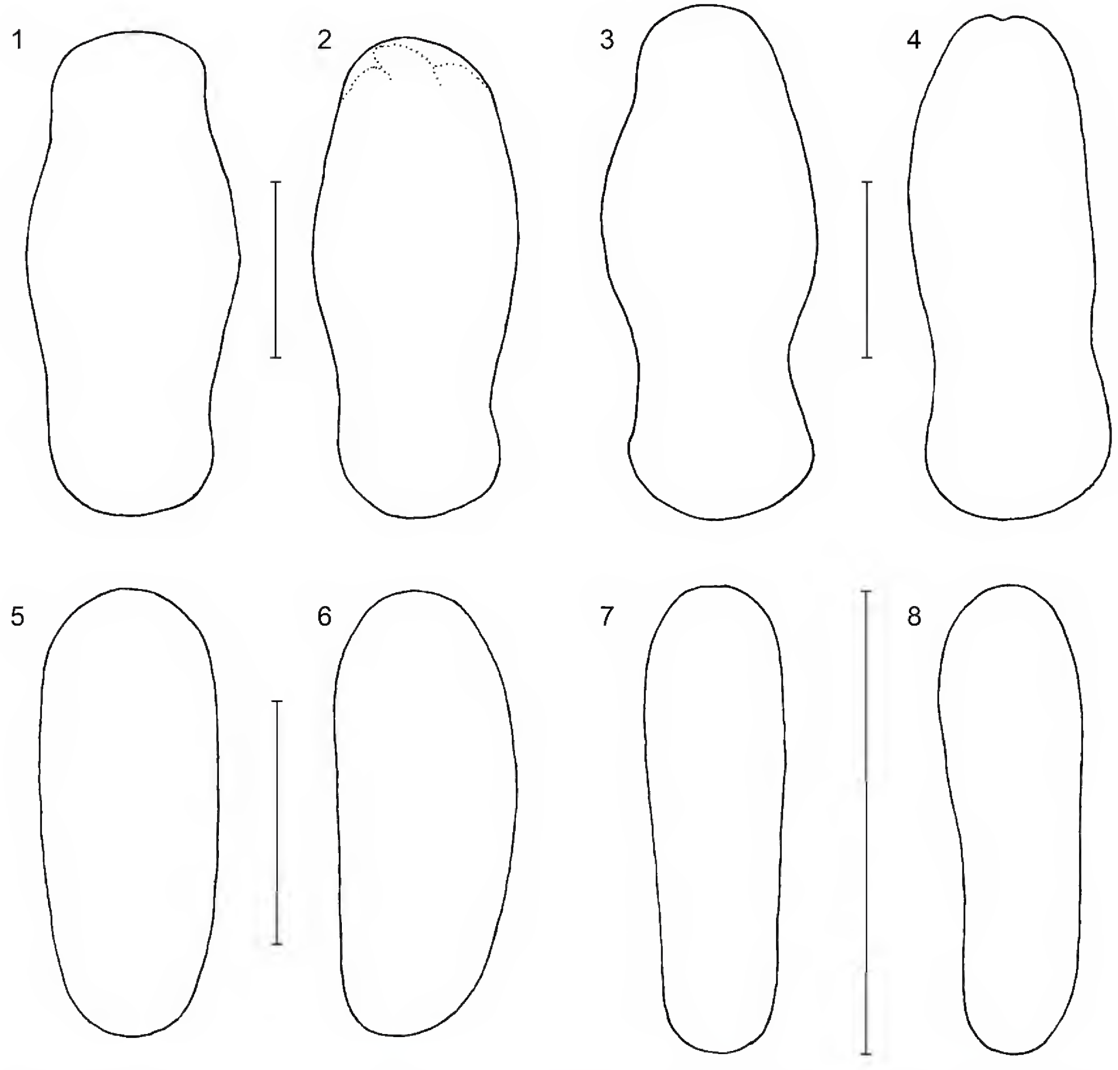
When viewed with SEM, chorion at anterior end of egg with strongly expressed, elevated hexagons that converge and narrow (figs. 15, 16) as they approach micropyle; micropyle circular, presumably with entrance pores situated peripherally around central plate (fig. 17); chorion at posterior end of egg smooth, lacking hexagonal ridges (fig. 18).

Material Studied: Four eggs: Panamá: Colón Prov. 4 km SE Sabanitas, Roubik Preserve, March, 2018 (D. Roubik).

## *Scaptotrigona pectoralis* (Dalla Torre)

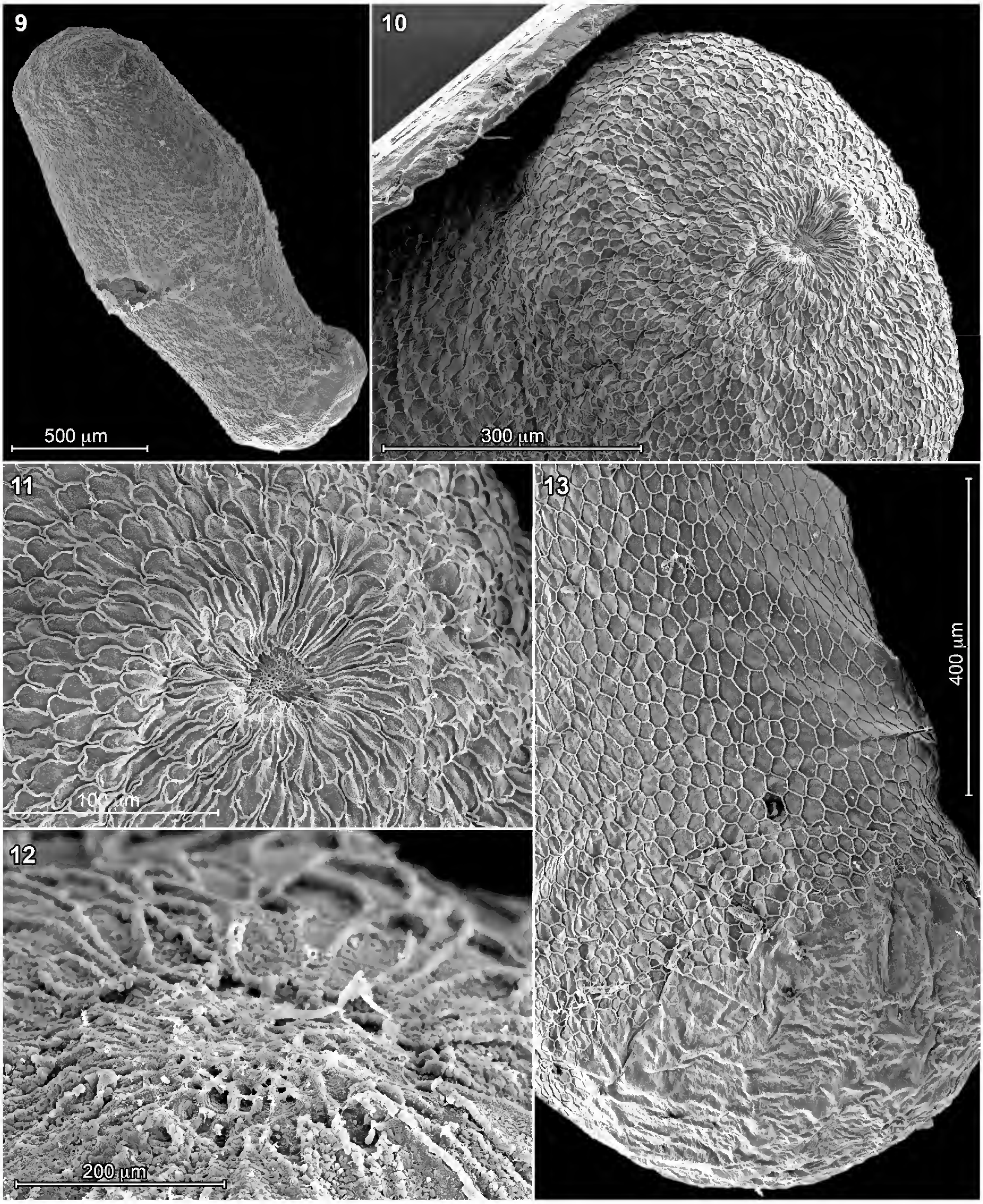
Figures 5, 6, 19–22

Diagnosis: The egg of *S. pectoralis* is somewhat oval in shape as seen in dorsal and ventral views (figs. 5, 6) and not linear (parallel sided) like that of *T. angustula*. It is also larger in size, but the anterior end is somewhat larger than the posterior end, as true for *T. angustula*.

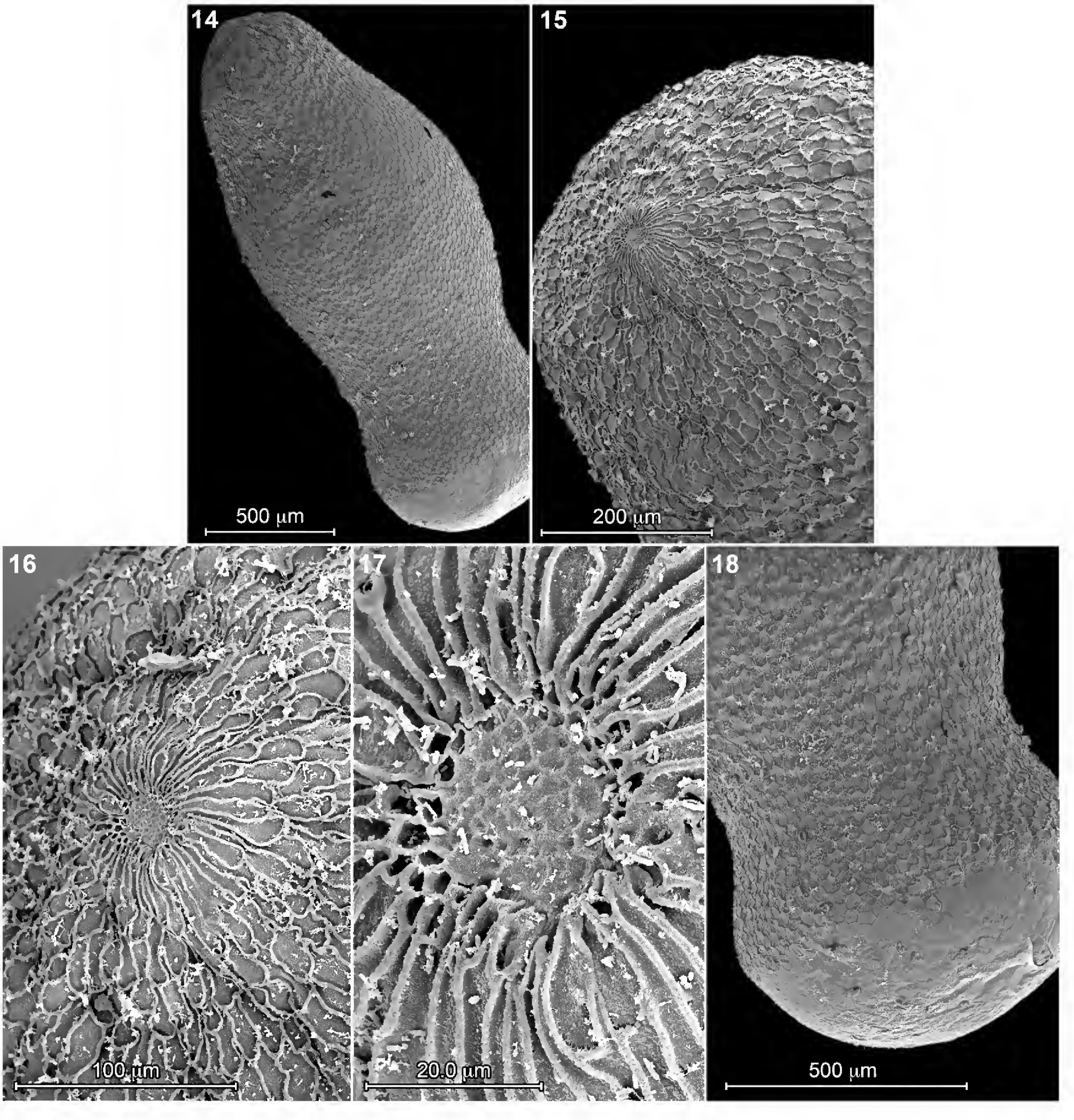



FIGURES 1–6. Diagrams of eggs of four species of Meliponini, anterior end to left. **1, 2.** *Melipona beecheii*, dorsal and side views. **3, 4.** *Melipona panamica*, presumed dorsal and side view. **5, 6.** *Scaptotrigona pectoralis*, dorsal and side views. **7, 8.** *Tetragonisca angustula*, dorsal and side views.

DESCRIPTION: Color white, nonlustrous; chorion smooth under low magnification; clear chorion on one damaged specimen with strongly expressed hexagonal surface pattern visible even with light microscope. Shape elongate oval in side view; anterior end somewhat larger than rear end; in lateral view (fig. 19) dorsal surface curving more than ventral surface, so that broadest point between 1/3–1/2 distant from front; presumed micropyle flush with surface (not projecting), appearing as small, rough integumental area at anterior end. Dimensions of better-preserved two of three specimens: 1.475, 1.45 mm long, 0.575, 0.625 mm maximum dorsal view width.




FIGURES 9–13. SEM micrograph of egg of *Melipona beecheii*. **9.** Entire egg, full length. **10.** Anterior end. **11.** Close-up of micropyle. **12.** Extreme close-up of micropyle. **13.** Posterior end showing lack of hexagonal patterning.




FIGURES 14–18. SEM micrographs of egg of *Melipona panamica*. **14.** Entire egg, full length. **15.** Anterior end, showing slightly bulging micropyle. **16, 17.** Close-ups of micropyle. **18.** Posterior end, showing loss of hexagons at extreme end.

When viewed with SEM, chorion at anterior end of egg with strongly expressed, elevated hexagons that converge and narrow (fig. 20) as they approach micropyle; micropyle circular with pores central (fig. 21); chorion at posterior end of egg lacking hexagonal ridges (fig. 22).

Material Studied: Two eggs with chorions (one still attached): Mexico: Yucatán: Xmatkuil, January 17, 2018 (J. Quesada). Microphotographs of live eggs (figs. 54–56).

## *Tetragonisca angustula* (Latreille)

Figures 7, 8, 23–26

Diagnosis: The egg of *T. angustula* (figs. 7, 8) is the smallest of the four species of meliponine eggs described here. It is linear in form with the posterior end narrower than the anterior end, as is the case with the slightly longer and more oval egg of *S. pectoralis* (figs. 5, 6).

Description: Color white; chorion faintly uneven when viewed at high magnification with stereomicroscope. Shape (figs. 7, 8) moderately elongate; when viewed from above or below, with sides converging slightly and symmetrically toward posterior end; long axis of egg perhaps slightly curved (possibly accident of preservation) in lateral view (fig. 8). Dimensions of 10 specimens selected at random: length 1.03 mm, range 1.0–1.05 mm, maximum width 0.35 mm, range 0.32–0.36.

When viewed with SEM, most of chorion with distinct pattern of elevated hexagons (fig. 23) but extreme posterior end smooth (fig. 27); micropylar area slightly bulging (fig. 24) with micropyle slightly recessed (fig. 25).

Material Studied: More than 10 eggs: Panamá: Colón Prov., 4 km SE Sabanitas, Roubik Preserve, March, 2018 (D. Roubik).

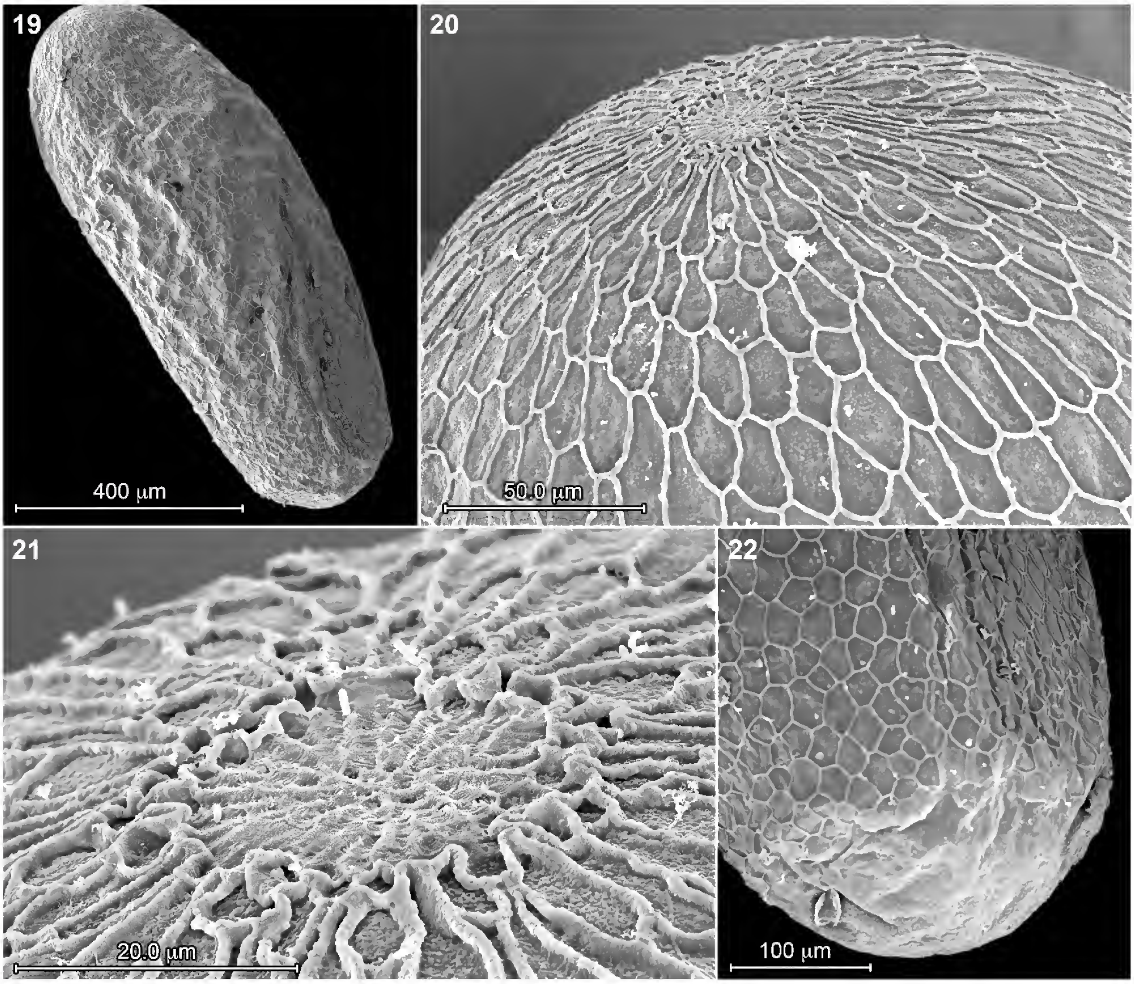
## Last Larval Instars of Meliponini

Frontal views of a larval head can vary depending on the tilt of the head capsule. To minimize this variability all head capsules were illustrated when each specimen was positioned so that its two hypostomal ridges were at right angles with the horizontal surface.

Rather than presenting a separate diagnosis for each taxon treated here, the following is a comparative diagnosis of the mature larvae of the four genera (five species) treated herein.

## Comparative Diagnosis of Mature Larvae of Meliponini

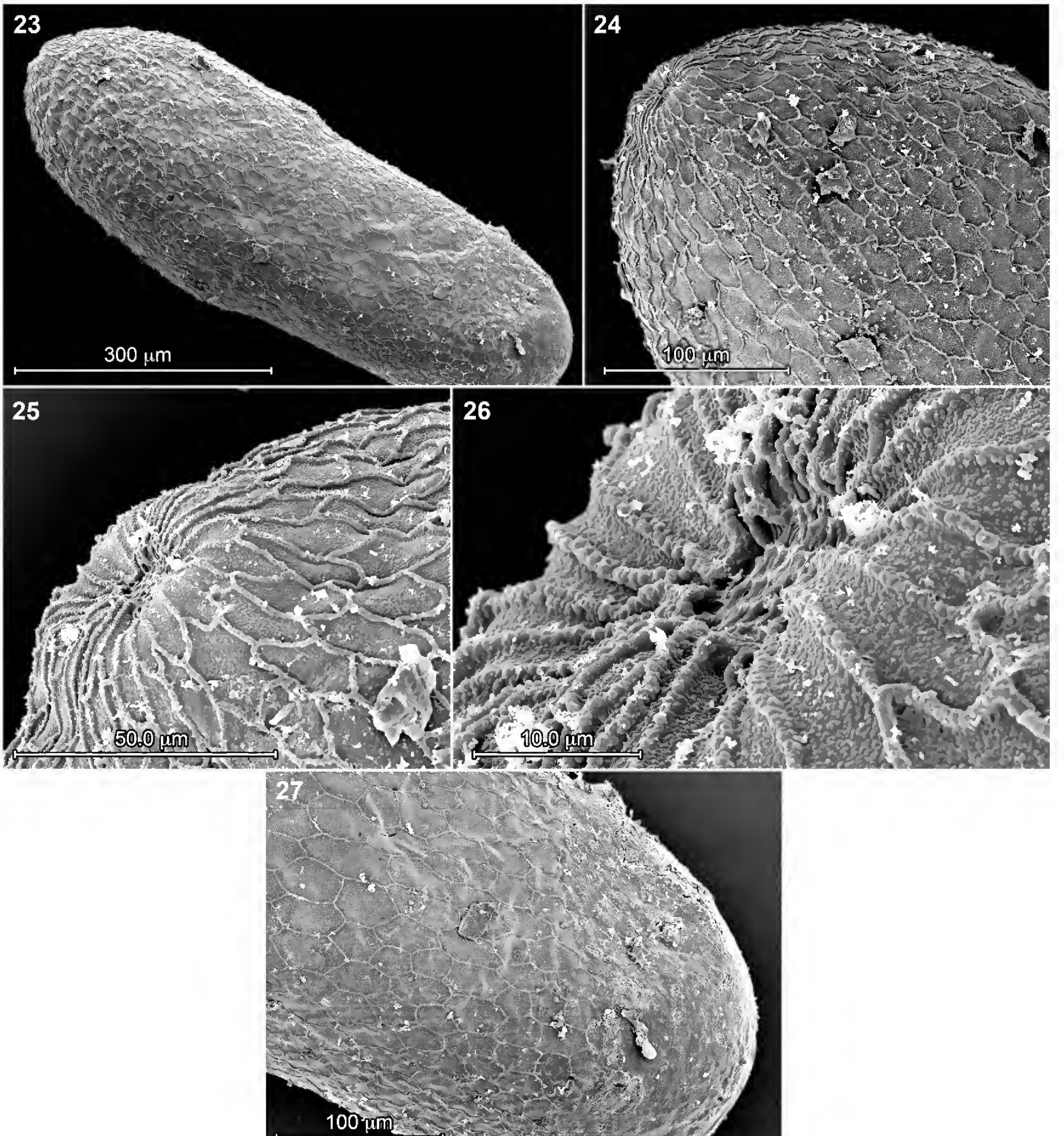
Recent studies of bee larvae have shown that, with the exception of *Apis*, mature larvae of corbiculate bees (i.e., Euglossini, Meliponini, Bombini, and Apini) can be recognized because their thoracic segments bear a dorsolateral pair of small but distinct conical tubercles. More recent investigations, in which cleared specimens have been stained with Chlorazol Black E, reveal the integument from which each tubercle arises is accompanied by an often large transversely oblong blue stain, indicating that the composition of the cuticle supporting the tubercle is different from the more flexible body cuticle elsewhere. With *Apis*, there are no tubercles of this sort, but the integument on which each would presumably have been found is somewhat raised and defined otherwise by a transversely oblong blue stain when treated with Chlorazol Black E (fig. 28). Interestingly, the paired, stained elevated areas are not only on the thoracic segments but also continue posteriorly at least as far as abdominal segment 2.




FIGURES 19–22. SEM micrographs of egg of *Scaptotrigona pectoralis*. **19.** Entire egg, full length, lateral view. **20.** Anterior end. **21.** Close-up of micropyle. **22.** Posterior end of egg showing lack of hexagonal patterning.

Many studies (e.g., Ritcher, 1933; Michener, 1953; Rozen et al., 2018a, 2018b) show that with *Bombus* the small, paired dorsolateral tubercles on each thoracic segment are characteristic for the genus. The same configuration of such tubercles holds for the known larvae of the Euglossini except for species of *Euglossa*, which in addition to the thoracic tubercles have a set of paired tubercles on the first abdominal segment (Rozen, 2016: fig. 17; 2018: figs. 18, 21, 22). Moreover, the current survey of select Meliponini shows that in addition to the paired dorsolateral tubercles on the thoracic segments and the first abdominal segment, these tubercles are also on other abdominal segments in many taxa. It also reveals that these tubercles and their cuticular platforms can vary in size and extent of staining, depending on the taxon.

The first two species examined, *M. fallax* and *M. trinitatis,* have the largest bodies and like *Euglossa* exhibit paired dorsolateral tubercles on the thoracic segments and the first abdominal segment. Since they lack an elongate prothorax (see figs. 29, 36) or tubercles on their vertices (figs. 32, 33), they would not be easily confused with *Euglossa* with its elongate, necklike prothorax (Rozen,




FIGURES 23–27. SEM micrographs of egg of *Tetragonisca angustula.* **23.** Entire egg, full length. **24.** Anterior end, slightly turned to view micropyle. **25, 26.** Close-ups of micropyle. **27.** Posterior end, showing loss of hexagons at extreme end.

2018: fig. 18) and paired tubercles on the vertex (Rozen, 2018: figs. 19, 20). Michener (1953: 1090) described the larva of *M.* (*Melipona*) *quadrifasciata* Lepeletier and claimed it had "minute conical dark and slightly sclerotized dorsal or dorsolateral tubercles" on the thoracic segments, but made no mention of such tubercles on the abdomen. Since abdominal dorsolateral tubercles often have far less pigmentation than their thoracic counterparts, he may—or may not—have overlooked them.

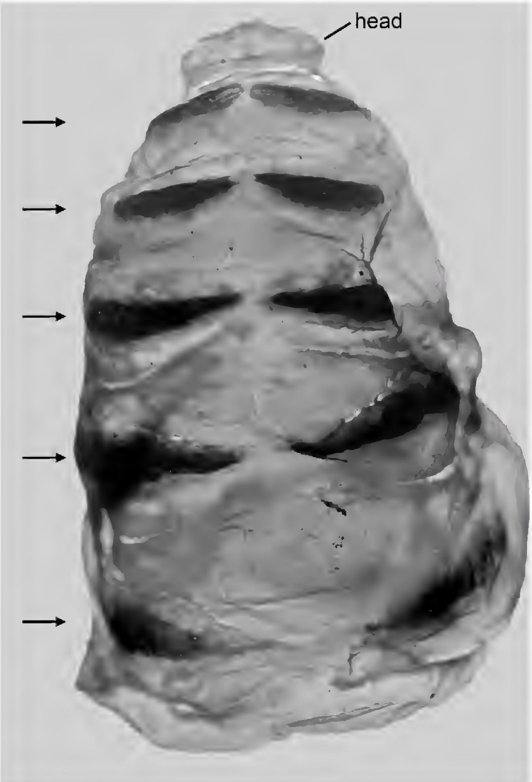



FIGURE 28. Microphotograph of anterior dorsal surface of cleared male last larval instar of *Apis mellifera* (anterior end at top) stained with Chlorazol Black E, showing (arrows) paired, transversely oblong dark areas on three thoracic segments and first two abdominal segments.

Michener's description and diagrams of the larva of *Partamona peckolti* (Friese) (Michener, 1953: figs. 266–271, as *Trigona* (*Partamona*) *cupira* (Smith)), a South American species and thus not studied by Michener (see also below), correspond closely with those presented for *Partamona musarum,* below, particularly with respect to placement of tubercles, mandibular shape, and general appearance. Only the illustration (Michener, 1953: fig. 274) of the spiracle with a seemingly elongate subatrium does not match the very short one of *P. peckolti.*

The other three available larval representatives of the Meliponini have a considerably smaller body size and many more abdominal body segments with paired dorsolateral tubercles. Of these three species, *Nogueirapis mirandula* (fig. 41) and *Partamona musarum* (fig. 44) have head capsules that, when viewed from the front, are not bilobed whereas the head of *Tetragonisca angustula* (fig. 51) is clearly broad and bilobed in frontal view.

Published accounts of other meliponine larvae by Lucas de Oliveira include the following: *Plebeia droryana* (Friese) (Lucas de Oliveira, 1965: figs. 1A, 3B); *Lestrimelitta limao* (Smith) (Lucas de Oliveira, 1968: figs. 1A, 3B); *Lestrimelitta ehrhardti* (Friese) (Lucas de Oliveira, 1968: figs. 1B, 3I); and *Leurotrigona muelleri* (Friese) (as *Hypotrigona* (*Leurotrigona*) *muelleri* (Friese)) (Lucas de Oliveira, 1970: figs. 1, 3B). The first illustration identified for each shows a uniformly thick-bodied larva with pairs of small dorsolateral tubercles on all body segments except for abdominal segments 9 and 10. The second illustration for each species is the larval mandible, inner view, with an extremely elongate, narrow to very narrow apex like that of *Partamona testacea* shown here (fig. 47). While these diagrams demonstrate the morphological homogeneity of the tribe, they do not help to distinguish one taxon from another.

## Last Larval Instar of *Melipona* (*Michmelia*) *fallax* Camargo and Pedro

Figures 29–35

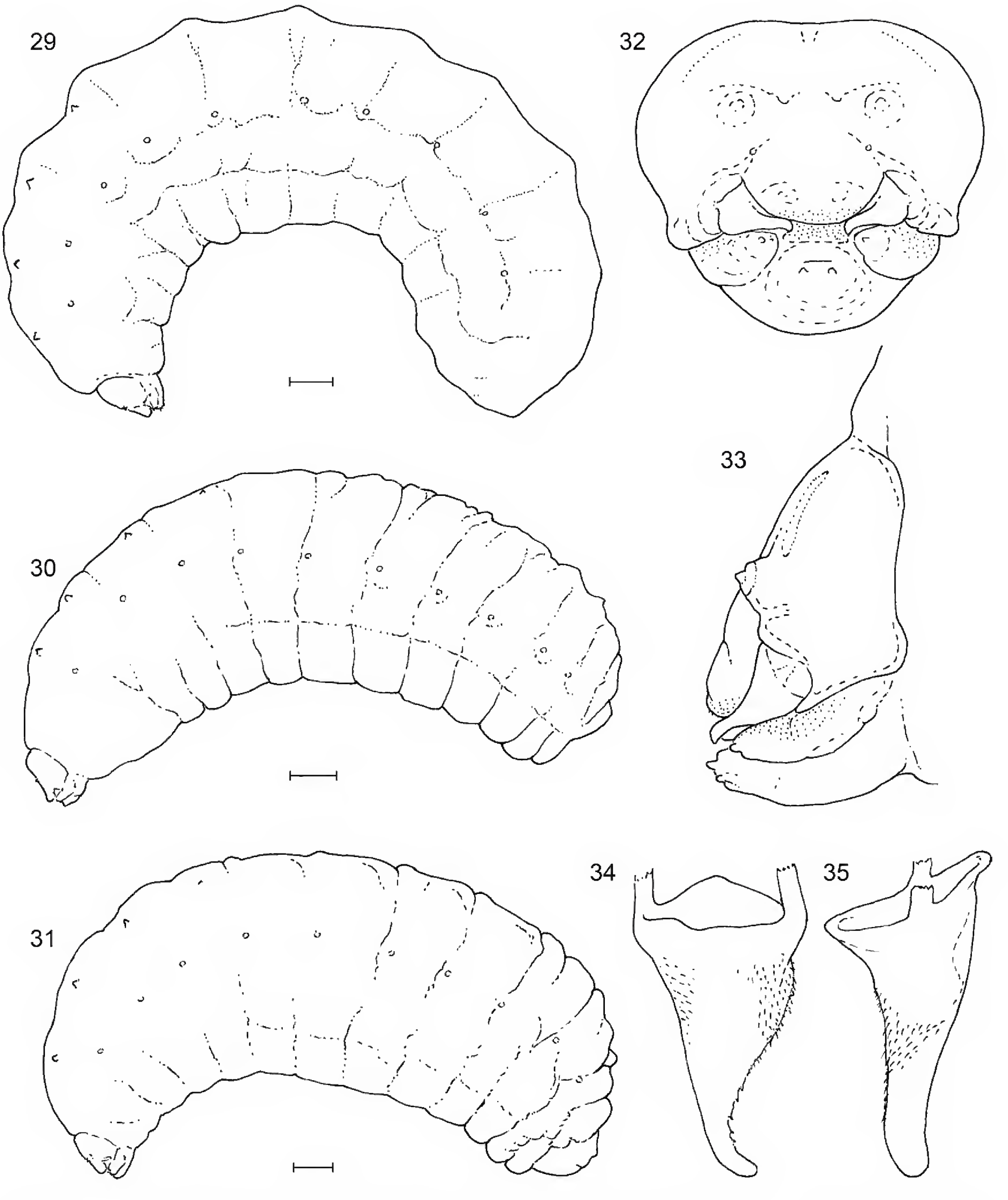
Except where stated otherwise, post- and predefecating stages of the last larval instar of this species are essentially identical.

Description: **Head** (figs. 31–34): Sclerotized integument of head capsule and mouthparts very faintly pigmented although thicker sclerotized areas slightly darker than thin areas; anterior tentorial pits darkly pigmented, as well as posterior mandibular articulation. Exposed surface of labrum unpigmented. Apex of maxilla faintly pigmented; articulating arm of stipes distinctly pigmented; maxillary palpus tending to be faintly pigmented. Prelabium bearing weakly pigmented sclerotic ring including narrow dorsal bridge; pigmentation of ring gradually diminishing toward venter; extreme labial apex unpigmented except for palpi and salivary lips; apex of labium lacking dark maculation internally on salivary duct behind salivary lips. Integument of head capsule and mouthparts with scattered moderately short setiform sensilla.

Head size very small relative to body size (figs. 29–31); front of head in lateral profile (fig. 33) relatively flat below narrow vertex, so that frons, clypeus, and labrum closely aligned and angling sharply from jutting labiomaxillary region in lateral view (fig. 33); head capsule moderately broad, but summit of vertex scarcely bilobed in frontal view (fig. 31). Tentorium complete at least on one specimen except tentorial bridge interrupted medially; posterior tentorial pits normal in position; posterior thickening of head capsule weakly developed, not bending forward medially as seen in dorsal view; coronal ridge only faintly evident at posterior thickening of head capsule, otherwise absent; epistomal ridge (fig. 32) evident on cleared head capsule from anterior mandibular articulation to anterior tentorial pit but fading out mesad of pit, although sclerotization of front of head mesad of pits faintly thicker; front of head capsule bearing pair of paramedian depressions at level of antennae. Parietal bands evident (figs. 32, 33). Antennal prominences, although weakly projecting in lateral view (fig. 33), well defined because sclerotized area around each depressed and basal ring of antennal ring projecting forward (fig. 33); area between antennal ring and antennal papilla membranous, so that degree of papilla projection variable; antennal papilla small, length, about as long as basal diameter, bearing perhaps three sensilla. Vertex narrowly rounded in lateral view; frontoclypeal area not projecting beyond labrum (fig. 33); labrum vaguely bilobed; apical surface densely spiculate.

Mandible (figs. 34, 35) only faintly pigmented, sometimes with dark area on apical edge; mandibular form elongate, tapering toward apex in all views, slender in inner (fig. 34) and outer views, with extreme apex curving adorally into weakly defined slender scoop-shaped apex to elongate concavity, its dorsal apical edge with small slender teeth toward base; base of concavity with large patch of extremely fine spicules; outer surface of mandibular base with pattern of evenly spaced spicules; mandibular apex evenly rounded, without teeth.

Labiomaxillary region produced and not greatly fused; pigmentation faint on both post- and predefecating forms, described above; labium projecting somewhat beyond maxilla in lateral view (fig. 33). Maxillary apex not bent mesad; palpus apical in position, short, only slightly longer than basal diameter, weakly pigmented; galea represented by small tubercle; galea projecting at maxillary apex, bearing several sensilla; stipes apically partly ringed by faint sclerotization; articulating arm of stipes pronounced, darkly pigmented; basal articulation of stipes to cardo faintly pigmented; membranous sides of maxilla finely, uniformly spiculate; dorsal and inner apical surface more densely spiculate, Strongly projecting labium divided into prementum and postmentum, and bearing apically projecting broad lips of slitlike salivary




FIGURES 29–35. Diagrams of last larval instar of *Melipona fallax*. **29.** Predefecating form, full length. **30.** Early postdefecating form. **31.** Postdefecating form. **32, 33.** Head, frontal and lateral views. **34, 35.** Right mandible dorsal and inner views.

opening, its width slightly shorter than distance between bases of labial palpi; length of labial palpus about equal to basal diameter. Hypopharynx a pronounced broad mound, as indicated in figure 32, spiculate.

**Body:** Dorsal integument from posterior margin of head to anus more or less densely covered with fine spicules to level of spiracles; spicules fading out below level of spiracles; density of spicules reduced along intersegmental lines; scattered minute setae, longer but narrower than spicules, present but less abundant than spicules.

Thoracic segments each with pair of small, pointed, apically pigmented tubercles present dorsolaterally on caudal annulet (figs. 29–31) as characteristic of corbiculate taxa; first abdominal segment also with pair of such tubercles, unlike those of *Bombus*; on cleared, stained specimen, each tubercle mounted in approximate middle of transversely oblong, more darkly stained area; although tubercles absent on other abdominal segments, paired, transversely oblong, darkly stained areas also appearing on abdominal segments 7 and 8, with weakly staining areas on 9. Body form of postdefecating larva (fig. 31) and that of predefecating larva (fig. 29) differing substantially in lateral view; postdefecating form more rotund, less linear with body segmentation less conspicuous and with lateral body swelling inconspicuously, in contrast to these features in predefecating form. Spiracles (figs. 36–39) small relative to body, with rim; atrial opening small relative to diameter of rim; radial width of peritreme about as wide as atrial opening; atrial wall smooth, without spines or distinct ridges; primary tracheal opening a simple rim slightly larger than atrial opening; subatrium short, consisting of 3–4 annulations; flexure collapsed, long.

Material Studied: More than 25 last larval instars: (a.k.a. *Melipona fuliginosa* Lepeletier) Panamá: Kuna Yala: El Llano–Carti Road (D. Roubik).

Discussion: The subatrium in the predefecating larva (fig. 37) is clearly annulated, but the annulations apparently become obscure later with subatrium collapsed (fig. 38).

## Last Larval Instar of *Melipona* (*Michmelia*) *trinitatis* Cockerell

Figure 39

Of the two specimens available, one was clearly a predefecating form as evidenced by the quantity of provisions in the midintestine. The other specimen contained only some residual provisions in the hind intestine suggesting that it was an early stage postdefecating form with body pigmentation also in the early development stage. Structurally, although significantly smaller in body size (as indicated), it was essentially identical to *M. fallax*, and so is not formally described here. However, it should be noted that it possesses paired dorsolateral tubercles on the thoracic segments as well as the first abdominal segment and the Chlorazol Black E staining pattern is identical to that *M. fallax*. Not surprisingly the pigmentation of the dorsolateral tubercles is reduced.

Material Studied: One postdefecating and one predefecating larvae: Trinidad: Nariva Swamp, April 08, 1964 (F.D. Bennett).

### Last Larval Instar of *Nogueirapis mirandula* (Cockerell)

Figures 40–43

Because of their small size and the limited number of specimens, the following description may appear less precise than other treatments here. One of the specimens had the hind gut filled with pollen, an indication that it was preserved before becoming a postdefecating form. The body integument of this specimen stained more reliably compared with two others that were cleared and stained.

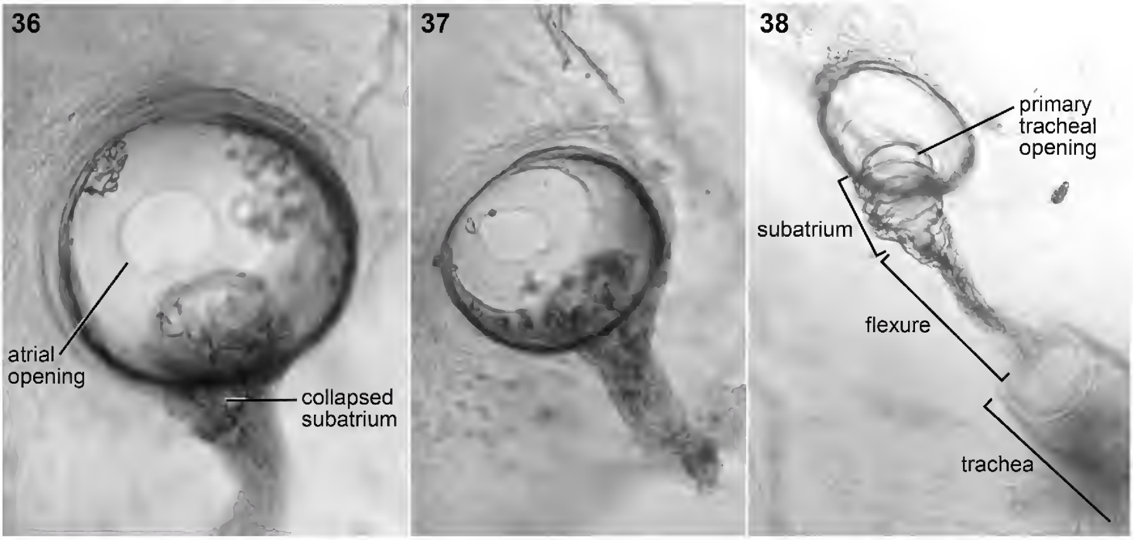
Description: **Head** (figs. 42, 43): Sclerotized integument of head capsule and mouthparts unpigmented except for mandibular apices, which are more or less faintly pigmented and indistinct marks along hypostomal ridge. Maxillary sclerites including articulating arm of stipes unpigmented though faintly staining. Labium unpigmented. Setiform sensilla of head capsule and mouthparts scarcely evident, usually shorter than minute protuberances bearing them.

Head size moderately small relative to body size (figs. 40, 41); front of head in lateral profile (fig. 43) gently curving from dorsal body surface and appearing to meet upcurving profile of labiomaxillary region; head capsule fairly broad in frontal view (fig. 42) but not bilobed. Tentorium incomplete at least on several specimens; posterior tentorial pits normal in position; posterior thickening of head capsule weakly developed dorsally but gradually strengthening toward posterior tentorial pits, not bending forward medially as seen in dorsal view; coronal ridge not evident; epistomal ridge (fig. 42) evident only as shallow furrow from upper end of weak pleurostomal ridge toward anterior tentorial pit, fading mesad of pits; middle of front of head capsule with shallow pair of paramedian depressions. Parietal bands weakly evident (figs. 42, 43). Antennal prominences, weakly defined; antennal papilla small, poorly defined, with length less than basal diameter, bearing perhaps three sensilla. Vertex narrow in lateral view; frontoclypeal area not projecting beyond labrum (fig. 43); labrum broad, vaguely bilobed; apical surface densely supplied with spicules.

Mandible faintly pigmented toward apex; form similar to that of *M. fallax* but narrower apically; extreme apex swelling somewhat before ending with spoon-shaped apical concavity; extreme apex bent sharply adorally in dorsal and ventral views, so as to appear hook shaped; surface of mandibular apex bearing fine structures, presumably spines.

Labiomaxillary region somewhat produced and not greatly fused; pigmentation nearly absent; apices of labrum, maxilla, and labium projecting equally in lateral view (fig. 43). Maxillary apex not bent mesad; palpus apical in position, short, about as long as basal diameter; galea represented by two small tubercles at maxillary apex; cardo and stipes well formed but scarcely pigmented; articulating arm of stipes evident, perhaps faintly pigmented; dorsal surface of maxilla and basal surface of prementum bearing coarse spicules. Prementum bearing apically projecting, slitlike salivary lips slightly shorter than distance between bases of labial palpi; length of labial palpus about equal to basal diameter. Hypopharynx presumably low mound bearing several spicules laterally.

**Body:** Dorsal integument from posterior margin of head to anus more or less densely covered with fine spicules to level of spiracles; spicules fading out below level of spiracles; density




FIGURES 36–38. Microphotographs of spiracles of *Melipona fallax*. **36.** Spiracles of postdefecating larva with circular atrial open and subatrium collapsed. **37.** Spiracle of postdefecating larva with elliptical atrial opening. **38.** Spiracle of predefecating larva with subatrium annulate, not collapsed, but with flexure collapsed and attached to tracheal system.

of spicules reduced along intersegmental lines; scattered minute setae present, longer but more slender and less abundant than spicules; spicules on paired dorsolateral swellings of most body segments large and those on posterior dorsal margins of abdominal segments 9 and 10 dense as well as large; spicules on dorsal body elsewhere abundant but small, often in series along transverse cuticular lines.

Thoracic segments each with pair of small, pointed, apically sclerotized, mostly unpigmented conical tubercles present dorsolaterally on caudal annulet (figs. 40, 41), as characteristic of most corbiculate taxa; most abdominal segments with paired tubercles, unlike in *Bombus*; on cleared, stained specimen, each tubercle occurring in approximate middle of transversely oblong, more darkly stained area; however, tubercles on abdominal segments 6–8, while darkly stained, becoming progressively less sclerotized and pronounced toward posterior end of abdomen; on abdominal segment 9 and 10, tubercles absent, but caudal elevation of each segment merging at midline, creating elevated, dorsally stained band on both segments (best observed on defecating larva, immediately anterior to pygidial ridge, as defined by Rozen (2018) (although integument encircled by ridge not darkly stained). Spiracles moderately small with atrial rim well identified as a circle although only questionably projecting beyond body wall; peritreme not detected though probably present; atrium shallow; atrial wall without spines, smooth except for faint concentric swellings; primary tracheal opening obviously simple rim with connection to flexure uncertain because subatrium not well sclerotized (examination of active last larval instar should help to identify connection of spiracle and tracheal system); flexure collapsed, long.

Material Studied: Four last larval instars: Panamá: Colón Prov.: 25 km NE Puerto Pilón, V-22-1986 (D. Roubik).

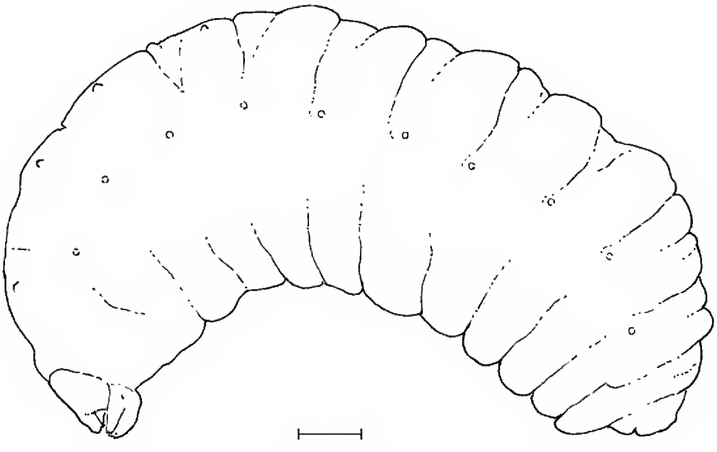


FIGURE 39. Early postdefecating larval instar of *Melipona trinitatis*, lateral view.

## Postdefecating Larva of *Partamona musarum* (Cockerell)

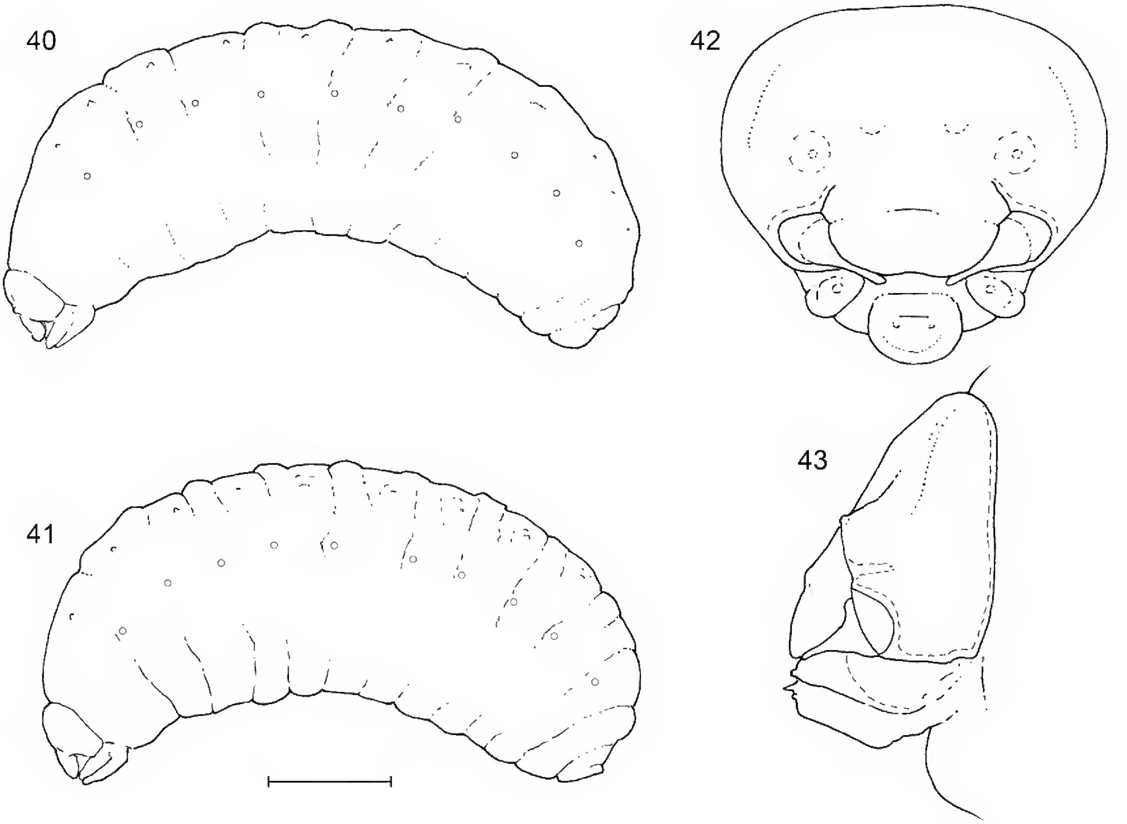
Figures 44–48

Description: **Head** (figs. 45, 46): Sclerotized integument of head capsule and mouthparts unpigmented except for mandibular apex, which is faintly pigmented. Sensory setae no longer setiform, reduced to sharp points on apices of scattered small swellings.

Head size small relative to body size (fig. 44); front of head in lateral profile relatively flat below narrow vertex, so that frons, clypeus, and labrum closely aligned and angling sharply from strong labiomaxillary region in lateral view (fig. 46); head capsule moderately broad; vertex not bilobed in frontal view (fig. 45). Condition of tentorium unknown; posterior tentorial pits normal in position; posterior thickening of head capsule narrow, not bending forward medially as seen in dorsal view; coronal ridge absent; epistomal ridge (fig. 45) evident from anterior mandibular articulation to anterior tentorial pit and beyond to level of middle of antennae before curving upward and ending; front of head capsule without noticeable pair of paramedian depressions at level of antennae. Parietal bands evident (figs. 45, 46). Antennal papilla mounted on projecting bulbous base forming distinct circle on front of head capsule (fig. 46); antennal papilla with length about as long as basal diameter, bearing perhaps four sensilla. Vertex narrowly rounded in lateral view; frontoclypeal area not projecting beyond labrum (fig. 46); labrum vaguely bilobed; apical surface densely spiculate.

Mandible (figs. 47, 48) faintly pigmented; mandibular form extremely slender in inner and outer views, with adorally curving shape; apex with weakly defined, slender, scoop-shaped apical concavity with shape accentuated by fine teeth around extreme apex.

Labiomaxillary region produced and not greatly fused; pigmentation absent; labium and maxilla ending equally in lateral view (fig. 46). Maxillary apex not bent mesad; palpus apical in position slightly longer than basal diameter; galea evident at maxillary apex, bearing several sensilla; articulating arm of stipes present; basal articulation of stipes to cardo faintly evident;

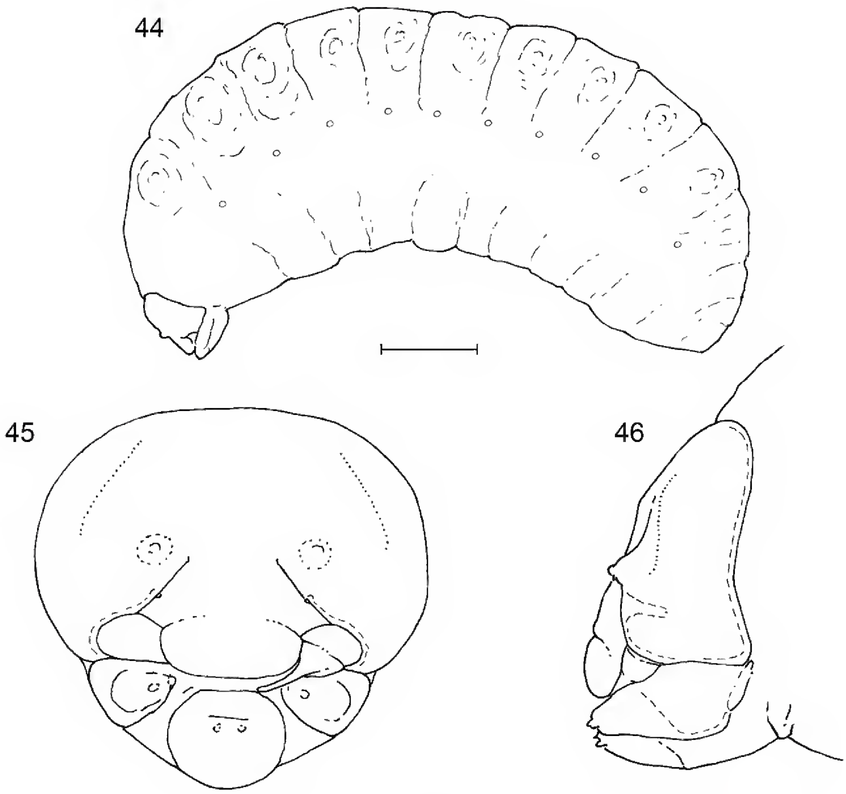



FIGURES 40–43. Diagrams of last larval instar of *Nogueirapis mirandula.* **40.** Defecating form, lateral view. 41. Postdefecating form, lateral view. 42, 43. Head, frontal and lateral views.

dorsal and inner apical surface of maxilla questionably spiculate. Labium divided into prementum and postmentum, bearing apically projecting broad lips of slitlike salivary opening, its width about same as distance between bases of labial palpi; length of labial palpus slightly shorter than basal diameter. Hypopharynx not visible.

**Body:** Dorsal integument from posterior margin of head to anus more or less densely covered with fine spicules that disappear well above level of spiracles; these spicules possibly intermixed with far less abundant minute setae.

Thoracic segments each with pair of slightly elevated, transversely oblong surfaces dorsolaterally on caudal annulet: each surface with small, weakly pointed, nipplelike nonpigmented tubercle (fig. 44): these surfaces taking on slightly characteristic sheen when stained with Chlorazol Black E; abdominal segment 1–6 also with similar elevated surfaces and tubercles (fig. 44), but these tubercles diminishing further posteriorly; paired, transversely oblong, darkly stained areas without tubercles also appearing on abdominal segments 7 and 8, with weakly stained areas on 9. Spiracles moderately small relative to body size (fig. 44), peritreme distinct; atrium shallow with concentric annulations but otherwise smooth; primary tracheal opening




FIGURES 44–46. Diagrams of postdefecating larva of *Partamona testacea.* **44.** Entire larval, lateral view. **45, 46.** Head frontal and lateral views.

a simple rim slightly larger than atrial opening; subatrium very short, so that flexure seems to connect almost directly to atrium; flexure collapsed into single, long narrow, collapsed tube.

Material Studied: Two postdefecating larvae: Costa Rica: Hamburg Farm, I-21-1938, Nest #33 (F. Nevermann). Adults det. H.F. Schwarz as *Trigona t. testacea* (Klug). That name no longer applies to any bee north of South America and, from the locality, the species must be *Partamona musarum* (Cockerell). Similarly, the *Partamona* studied by Michener (1953) can only have been *Partamona peckolti* (Friese); see Moure et al. (2007).

## Predefecating Last Larval Instar of *Tetragonisca angustula* Latreille

Figures 49–52

Because only predefecating larvae of *T. angustula* were available, the reader should be aware that information on degree of natural pigmentation of larval structures reported here will be unreliable for comparison with postdefecating specimens of this or other meliponine species.

Description: **Head** (figs. 51, 52): Only mandibular apices faintly pigmented. Sensilla mostly small hemispherical projections with apical spike.

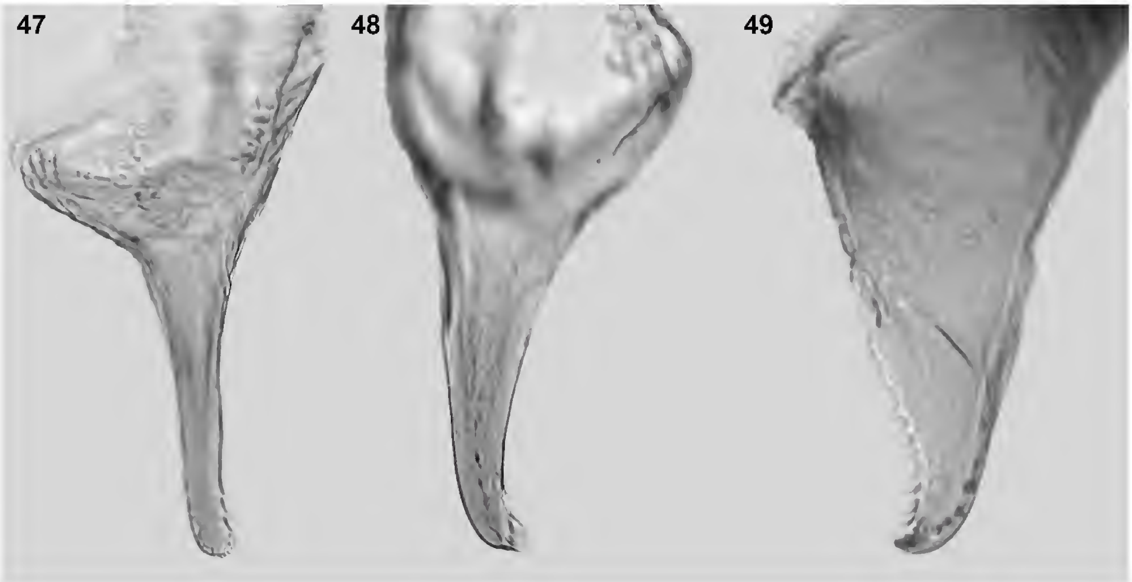
Head size very small relative to body (figs. 50); front of head in lateral profile (fig. 52) relatively flat below vertex, so that frons, clypeus, and labrum closely aligned and angling moderately with labiomaxillary region in lateral view (fig. 52); head capsule broad and summit of vertex bilobed in frontal view (fig. 51). Tentorium complete on one specimen, tentorial bridge interrupted medially on another specimen; posterior tentorial pits normal in position; posterior thickening of head capsule well developed except near vertex, where posterior margin of head capsule bends forward medially forming very broad V-shaped margin as seen in dorsal view; coronal ridge not evident; epistomal ridge (fig. 49) evident on cleared head capsule from anterior mandibular articulation to anterior tentorial pit, absent mesad of anterior tentorial pits; front of head capsule bearing only indistinct pair of paramedian depressions at level of antennae. Parietal bands evident (figs. 49, 50). Antennal prominences pronounced, but each restricted to base of its conspicuous projecting antennal papilla (fig. 50), bearing perhaps three sensilla. Vertex narrowly rounded in lateral view; frontoclypeal area not projecting beyond labrum (fig. 50); labrum vaguely bilobed; apical surface densely spiculate.

Mandibular form in dorsal or ventral view broad at base tapering to slender apex curving adorally; in inner or outer views, mandible moderately slender, tapering gradually and evenly to somewhat more sclerotized, nearly transparent, scoop-shaped apical concavity with dorsal and ventral apical edges bearing numerous sharp, apically directed teeth.

Labiomaxillary region produced and not greatly fused; pigmentation faint, absent on predefecating form; labium projecting little beyond maxilla in lateral view (fig. 50). Maxillary apex not bent mesad; palpus apical in position, moderately long, slightly longer than twice basal diameter; galea represented by small tubercle projecting at maxillary apex, bearing several sensilla; articulating arm of stipes darkly staining membranous sides of maxilla nonspiculate; dorsal and inner apical surface weakly spiculate. Projection of labium divided into prementum and postmentum not clearly defined, suggesting lack of retraction of prementum into postmentum; lips of slitlike salivary opening about as wide as distance between bases of labial palpi; length of labial palpus nearly twice as long as basal diameter. Hypopharynx unknown.

**Body:** Dorsal integument from posterior margin of head to anus more or less densely covered with fine spicules; spicules fading out above level of spiracles; scattered minute setae also detected.

Thoracic segments each with pair of small, pointed, apically nonpigmented tubercles dorsolaterally on caudal annulet (fig. 48), as characteristic of corbiculate taxa; also, abdominal segments 1–8 each with pair of tubercles in same relative position on their segments, but these tubercles becoming smaller and less apparent from abdominal segments 1 to 8, so that toward end of abdomen they nearly disappear; each tubercle of thorax and abdomen borne on transparent, transversely oblong area of integument that, when stained with Chlorazol Black E, becomes blue, so that presence and position of tubercles easily identified; transversely oblong stained areas of thoracic tubercles large; those of first abdominal segment much smaller with those of remaining segments even smaller, all as shown in figure 50; abdominal segment 9 without tubercles, but, when stained, transverse band runs along caudal annulet; abdominal segment 10 with semicircular stained band immediately in front of pygidial ridge from one




FFIGURES 47, 48. Microphotograph of right mandible of mature larva of *Partamona testacea*, **47.** inner view, showing markedly elongate, narrow apex, and **48.** Same, except slightly rotated to reveal apical scooplike shape. FIGURE 49. Microphotograph of right mandible of mature larva of *Tetragonisca angustula*, oblique inner view, demonstrating strongly spined dorsal and ventral apical edges and scooplike apex that is elongate but less so than that of *P. testacea*.

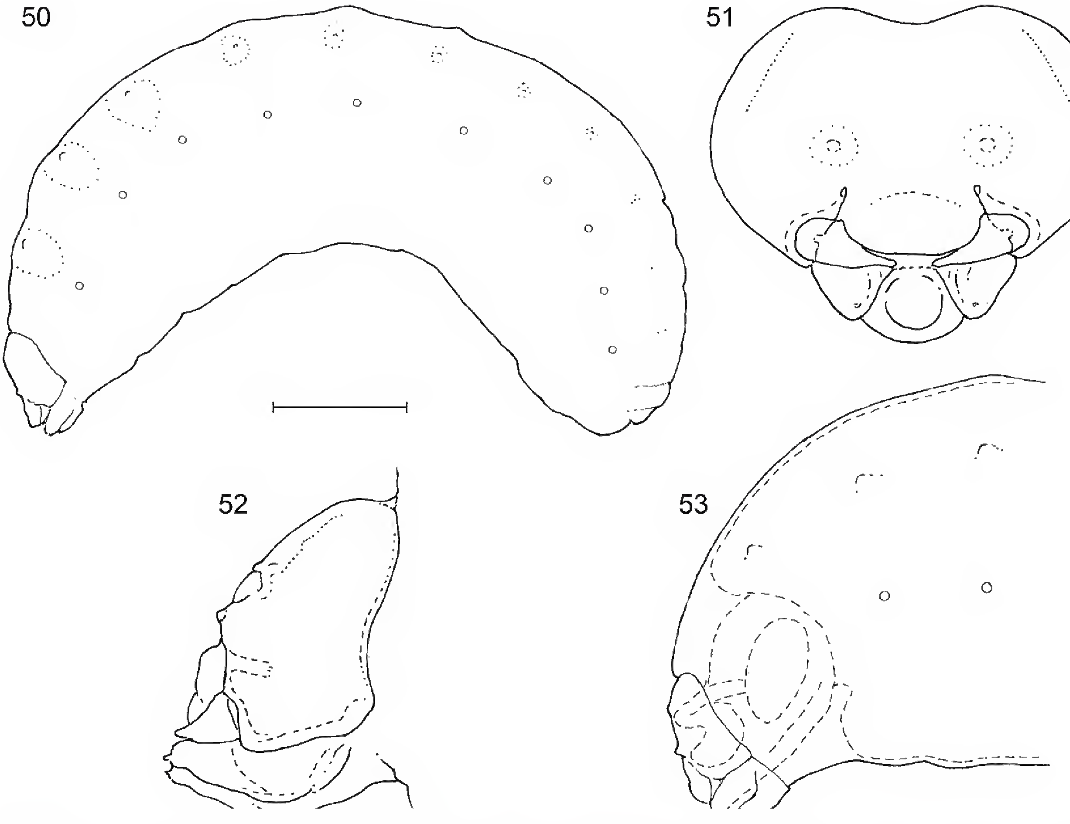
side of anus to the other. Spiracles small relative to body size (fig. 50), but thoracic spiracular rims tend to be largest while abdominal spiracles decrease in size posteriorly; atrial rim present; peritreme nearly transparent, difficult to observe; atrial wall without spines but faintly concentrically ringed; primary tracheal opening a simple rim slightly larger than atrial opening; subatrium moderately short, consisting of 3–4 annulations; flexure collapsed in postdefecating larva, partly open in predefecating larva.

Material Studied: More than four predefecating last instars: Panamá: Colón Prov. 4 km SE Sabanitas, Roubik Preserve, March, 2018 (D. Roubik).

## DISCUSSION

Although the samples of meliponine eggs described here are limited to only three genera, they suggest that eggs of the tribe tend to be more or less symmetrical along their long axis with the anterior end often tending to be slightly wider than the posterior end except for some species[4] of *Melipona*. Consistent with all known eggs of corbiculate bees is the strongly produced hexagonal surface pattern covering most of the chorion of reproductive eggs, with the

[4] It should be noted that although *M.* (*Melikerria*) *beecheii* (figs. 1, 2) and *M.* (*Michmelia*) *panamica* (figs. 3, 4) have a distinctive shape toward the anterior end of their eggs, the egg of *M.* (*Eomelipona*) *bicolor bicolor* Lep. (Velthuis and Velthuis, 1998: fig. 1a–d) appears to be more similar in shape to those of eggs of the other two species pictured herein (figs. 5–8).




FIGURES 50–52. Diagrams of predefecating last larval instar of *Tetragonisca angustula*. **50.** Predefecating larva, lateral view, with outline of stained areas indicated by dots. **51, 52.** Head frontal and lateral views. FIGURE 53. Diagram of head and thorax of *Partamona testacea* showing (dashed lines) position of developing pupal head, head appendages, and thorax within larval exoskeleton.

hexagons narrowing toward the micropyle and perhaps serving as spermatozoa guides leading to the openings of the micropyle. Also, considering all known eggs, their posterior ends lack any hexagonal patterning, suggesting the possibility that this is a functional specialization allowing the collapse of the rear end of the egg as the emerging larva disengages itself through the front of the chorion. The hexagonal reticulation of eggs in *Melipona* is typical of queen eggs and reproductive eggs of workers but not of trophic eggs laid by workers (Pereira et al., 2006).

In the current study dealing with functional anatomy of meliponine eggs, sampling has been inadequate to determine whether hatching spines exist on emerging larvae among any of the included taxa (Rozen, 2017).

In the genus *Melipona,* reports have shown that the biophysical properties of the chorion allow the egg to assume a vertical position on the surface of the liquid provisions (Velthuis and Velthuis, 1998; Velthuis et al., 2003). They posit that the smooth hydrophilic posterior surface and hydrophobic reticulations that nearly cover the remainder of the egg are the leading factors in keeping the egg upright. However, we hypothesize that the peculiar shape of the eggs in the genus (figs. 1–4, 9, 14) may be an additional specialization that tends to stabilize the egg in a

vertical position on the unstable provisions that can be described as "soupy." The swelling of much of the egg's contents in its midsection and the somewhat expanded posterior creates a lower center of gravity This makes the egg less likely to topple over than if it were a simple, uniformly slender form like that of *T. angustula,* which is quite firmly planted in a vertical position on the pastelike food mass. The consistency of provisions is similar in *Tetragonisca* and Euglossini (*Eulaema, Eufriesea,* and the *Euglossa* studied), unlike the provisions of *Melipona* (D. Roubik, personal obs.).

As in the other two genera discussed, the egg of *S. pectoralis* is also found upright with only the smooth posterior of the chorion in contact with the provisions (figs. 54–56). As these provisions share the same liquid consistency as those of *Melipona* and their eggs maintain a vertical orientation, one may infer that the chorion has a similar biophysical makeup (i.e., a hydrophilic base and lipophilic reticulations). The shape of the egg of *S. pectoralis* (figs. 5, 6) seems quite different from those of *Melipona* (figs. 1–4). However, if the large, unusual shape of the egg of *Melipona* is a specialization that lowers the center of gravity, thereby stabilizing the upright position of the egg, might the broad oval shape with a maximum length less than 2.5 times the maximum width possibly provides adequate stability in the case of *S. pectoralis*?

This study has not shed light on the number of larval instars involved with the meliponine life cycle. However, Lucas de Oliveira (1960) convincingly documented five instars in *Melipona* (*Eomelipona*) *bicolor schencki* Gribodo (as *Melipona nigra schencki* Gribodo).

One of the larval specimens of *Partamona* had developed sufficiently, so that its pupal features were visible within the larval exoskeleton (fig. 53). The pupal head capsule and front of the pupal prothorax occupied the larval prothorax while most of the pupal antennae and mouthparts resided in the larval head. It seems likely that these developmental features account for the robust and not elongate thoracic silhouettes of known tribal members as demonstrated in table 1.

Information concerning mature larvae is limited to seven of the 22 genera assigned to the Meliponini (listed by Michener, 2007), although the current trend in meliponine classification is to recognize at least 60 genera (Camargo and Pedro, 2007). Previous research suggests that as mature larvae, they range in size from very small to only moderate in size. To the extent known, all are robust forms with a small head attached to a robust thorax and abdomen. Spiracles characteristically seem to have a moderately shallow, smooth to faintly annulated atrial wall with a peritreme and a short subatrium that connects to a reasonably long flexure. Perhaps significant is the fact that the primary tracheal opening is a simple circular aperture

FIGURES 54–56. Microphotographs of cells of *Scaptotrigona pectoralis* (all from above) demonstrating liquid nature of provisions and orientation of eggs during development. **54.** Cells, some of which have been manually opened to inspect provisions, eggs, and live larvae; note reflective surface of provisions testifying to their liquidity. Top and bottom arrows point to eggs; two arrows on left point to curled larvae, partly submerged on one side while feeding on provisions; content of open cell without arrow uncertain. **55.** Close-up of two cells, one with recently deposited egg (below), the other (above) with egg nearly hatching. **56.** Even closer view of most developed egg in which white circular head of first instar can be distinguished from darker, faintly yellowish larval body, seen through transparent chorion, all indicating that egg remains vertical to surface of provision during embryogenesis with anterior end up.

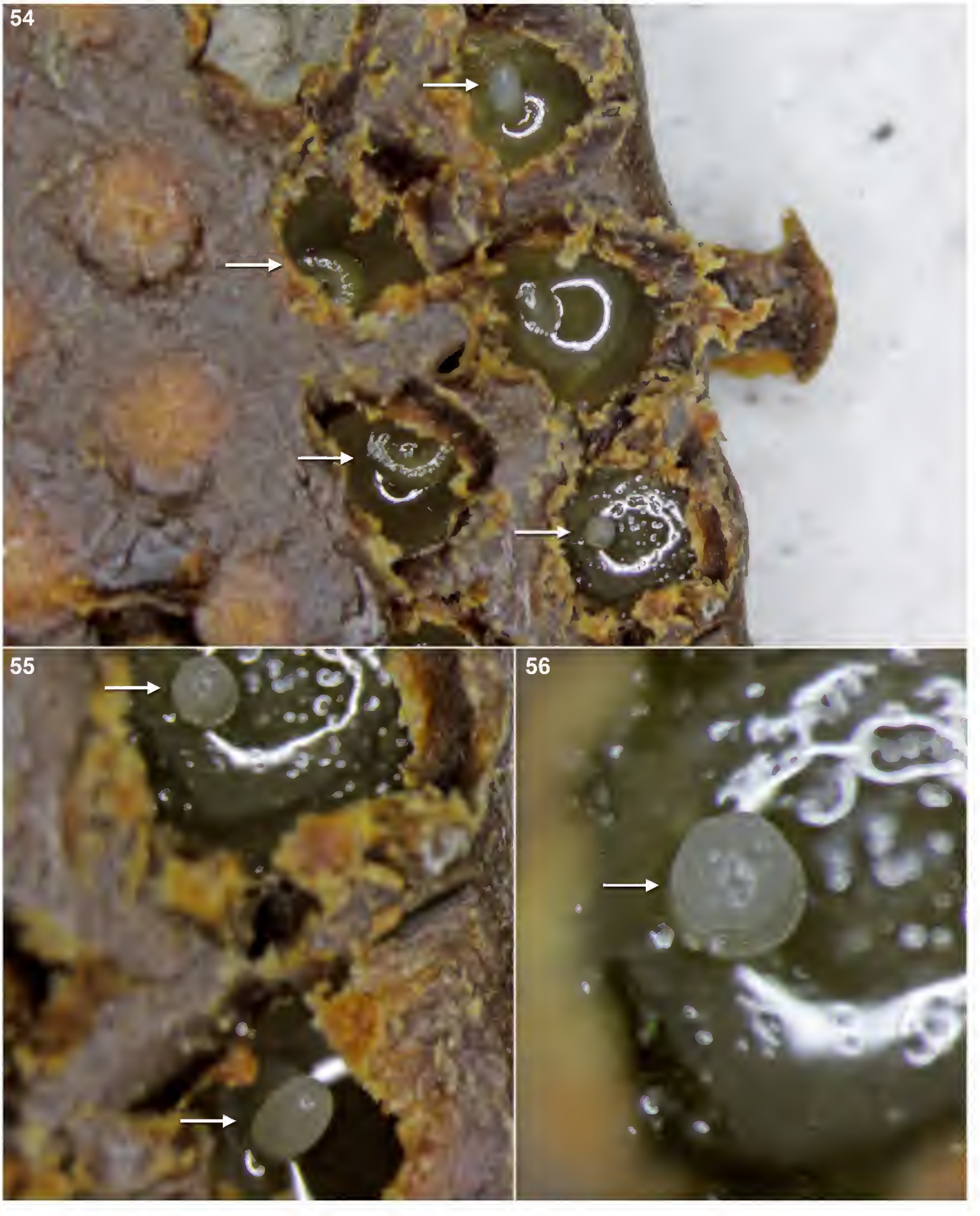

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TABLE 1. Illustrations of body shape, lateral view, of last stage larvae of the Meliponini.

| Taxon | Source |
|---|---|
| *Lestrimelitta limao* (Smith) | Lucas de Oliveira, 1968: fig. 1A |
| *Lestrimelitta ehrhardti* (Friese) | Lucas de Oliveira, 1968: fig. 1B, C |
| *Melipona* (*Michmelia*) *fallax* Camargo and Pedro | Herein: figs. 29–31 |
| *Melipona* (*Michmelia*) *trinitatis* Cockerell | Herein: fig. 39 |
| B*Nogueirapis mirandula* (Cockerell) | Herein: figs. 40, 41 |
| *Partamona cupira* (Smith) | |
| (as *Trigona* (*Partamona*) *cupira* Smith) | Michener, 1953: fig. 266 |
| *Partamona testacea* (Klug) | Herein: fig. 44 |
| *Plebeia droryana* (Friese) | Lucas de Oliveira, 1965: fig. 1 |
| *Tetragonisca angustula* (Latreille) | Herein: fig. 50 |
| *Trigonisca muelleri* (Friese) | |
| (as *Hypotrigona* (*Leurotrigona*) *muelleri* (Friese)) | Lucas de Oliveira, 1970: fig. 1 |

without spines or other ornamentation. The current study demonstrates that the anatomical structure of larval spiracles of the Meliponini and Euglossini seem nearly identical and very different from those of Bombini, which have extensively ornate atrial walls and primary tracheal openings (e.g., Ritcher, 1933: figs. 13–33: Michener, 1953: figs. 253, 254; Rozen et al., 2018b: fig. 61). It will be interesting to learn what that will tell us about the interrelationships of the involved tribes.

Although larval features of the Meliponini can be found among some or even most of the other corbiculate tribes (Euglossini, Bombini, and Apini), the single known unique larval feature of the Meliponini is the shape of its mandible. The last larval instar of Bombini displays a short robust, darkly pigmented, scoop-shaped, acutely pointed mandibular apex (Michener, 1953: figs. 251, 252); Rozen et al., 2018a: figs 34–36), similar to the Euglossini (Rozen, 2016: figs. 9–11, 20, 22; Rozen, 2018: figs. 23, 24), whereas the mandible of the Apini (*Apis*) is short, bluntly pointed, and lacking an apical concavity (Michener, 1953: figs. 278, 279). In contrast to all of these, the mandible of meliponines, where known, is at most weakly pigmented, apically elongate, and tapering strongly from its base to a long apical region as it curves adorally, with the dorsal and ventral edges nearly parallel before ending as a narrow, rounded scoop-shaped apex (Michener, 1953: figs. 267, 268, 271, 272; Lucas de Oliveira, 1960: fig. 2; 1965: fig. 3; 1968: fig. 3: A–C, H–J; 1970: fig. 3; herein, figs. 34, 35, 47, 48).

As pointed out at the start of Comparative Diagnosis of Mature Larvae of Meliponini, corbiculate mature larvae with the exception of *Apis mellifera* have small but distinct paired conical dorsolateral tubercles on some body segments, and all corbiculate larvae including those of *A. mellifera* have paired, dorsolaterally transverse patches of integument on some body segments that displays a characteristic dark blue stain when treated with a solution containing Chlorazol Black E (see those of *Apis mellifera*, fig. 28). Depending on the taxon, the paired tubercles and patches may be confined to the thoracic segments alone; to them and the first abdominal segment; or to additional abdominal segments. Their presence or lack thereof

implies a function currently not understood. Observations of live larvae in cells might offer interesting explanations.

This study has revealed little concerning the hatching process among corbiculate taxa. An earlier study (Rozen et al., 2017) indicated that in certain groups of bees, hatching spines along the sides of first instars function to enable them to emerge from their chorion. Since then, the absence of such spines (as well as the lack of one larval instar) was noted in a species of *Bombus* (Rozen et al., 20I8b), but there was little information from the current study regarding this matter. Although outstanding works dealing with the natural history of the Meliponini (e.g., Roubik, 1989) are available, there is still much to learn about the functional anatomy and developmental biology of their immature stages.

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Technician Humberto Moo, Universidad Autónoma de Yucatán, was responsible for the excellent microphotographs (figs. 54–56) of the deposited eggs and liquid provisions of *S. pectoralis.*

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